Cardiovascular response to group I metabotropic glutamate receptor activation in NTS

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Foley, C. Michael, Helen W. Vogl, Patrick J. Mueller, Meredith Hay, and Eileen M. Hasser. Cardiovascular response to group I metabotropic glutamate receptor activation in NTS. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1469–R1478, 1999.—Glutamate is the proposed neurotransmitter of baroreceptor afferents at the level of the nucleus tractus solitarius (NTS). Exogenous glutamate in the NTS activates neurons through ionotropic and metabotropic glutamate receptors (mGluRs). This study tested the hypothesis that group I mGluRs in the NTS produce depressor, bradycardic, and sympathoinhibitory responses. In urethane-anesthetized rats, unilateral 30-nl microinjections of the group I-selective mGluR agonist, 3,5-dihydroxyphenylglycine (DHPG) into the NTS decreased mean arterial pressure, heart rate, and lumbar sympathetic nerve activity. The dose of drug that produced 50% of the maximal response (ED50) was 50–100 μM. The response to microinjection of equal concentrations of DHPG or the general mGluR agonist 1-aminocyclopentane-1,3,5-tricarboxylic acid (ACPD) produced similar cardiovascular effects. The cardiovascular response to injection of DHPG or ACPD was abolished by NTS blockade of mGluRs with α-methyl-4-carboxyphenylglycine (MCPG). Blockade of ionotropic glutamate receptors with kynurenic acid did not attenuate the response to DHPG or ACPD injection. These data suggest that DHPG and ACPD activate mGluRs in the NTS and do not require ionotropic glutamate receptors to produce their cardiovascular response. In the NTS, the group I mGluRs produce responses that are consistent with excitation of neurons involved in reducing sympathetic outflow, heart rate, and arterial pressure.

sympathetic nerve activity; arterial baroreflex; 3,5-dihydroxyphenylglycine; 1-aminocyclopentane-1,3,5-tricarboxylic acid

ARTERIAL BARORECEPTOR afferents terminate in the nucleus tractus solitarius (NTS). The majority of evidence suggests that these baroreceptor afferents release glutamate as the primary neurotransmitter (2, 21). Glutamate receptors have been divided into two major categories: ionotropic glutamate receptors and metabotropic glutamate receptors (mGluRs). In the NTS, blockade of the ionotropic glutamate receptors with kynurenic acid abolishes arterial baroreflex function (28, 31, 37). However, combined antagonism of ionotropic glutamate receptors and mGluRs is required to block the response to exogenous glutamate in the NTS (12, 28, 31, 37). Furthermore, microinjection of the general mGluR agonist 1-aminocyclopentane-1,3,5-tricarboxylic acid (ACPD) into the NTS produces cardiovascular responses similar to glutamate (12, 31). These data confirm that mGluRs in the NTS can be activated to produce cardiovascular responses, and they may be involved in cardiovascular reflex function.

At least eight mGluR subtypes plus several splice variants have been identified, and they can be categorized into group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3), and group III (mGluR4, mGluR6, mGluR7, and mGluR8) on the basis of molecular homology and pharmacological profiles (7, 13). In a variety of systems, the mGluRs have been demonstrated to modulate synaptic transmission and are involved in long-term potentiation and long-term depression (1, 5, 7). The group I mGluRs have been described predominantly as postsynaptic and to have an excitatory effect on neurons (13). In contrast, the group II and III mGluRs are primarily presynaptic and exert an inhibitory effect on neurotransmission (13).

ACPD has been shown to activate most of the currently identified mGluRs (7), but it is unknown which group of mGluRs is responsible for its effects in the NTS. Microinjection of ACPD into the NTS decreases arterial pressure, heart rate, and lumbar sympathetic nerve activity (12, 31). This effect of ACPD is consistent with activation of neurons in the NTS that are involved in baroreflex inhibition of heart rate and sympathetic outflow. Specific mGluRs from all three groups (mGluR1α, mGluR2, mGluR5, and mGluR7) have been identified in the NTS (24). In the NTS, only mGluR1α appears to be localized to cell bodies, with the other mGluRs situated on processes and fibers (24). Given the general postsynaptic and excitatory effects of group I mGluRs in other systems and their location on cell bodies in the NTS, it seems likely that activation of this group of mGluRs is involved in the cardiovascular response to NTS injection of ACPD. Recently, 3,5-dihydroxyphenylglycine (DHPG) has been demonstrated to be a specific group I mGluR agonist that is devoid of actions at group II/III mGluRs (16, 26, 33). In other studies, DHPG has been used to investigate the actions of group I mGluRs in the regulation of neuronal excitability and synaptic transmission (11, 15, 16). The purpose of the present study was to determine the effects of selective activation of group I mGluRs in the NTS. We hypothesized that activation of group I mGluRs produces depressor, bradycardic, and sympathoinhibitory effects consistent with excitatory responses in NTS neurons.

METHODS

Experimental preparation. All procedures were approved by the Institutional Animal Care and Use Committee of the University.

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University of Missouri-Columbia. Adult, male Sprague-Dawley rats (n = 44) weighing 352 ± 7 g were anesthetized with urethan (1.2–1.5 g/kg ip). The right femoral artery and vein were cannulated (PE-10 tubing fused to PE-50) for measurement of arterial pressure and administration of drugs, respectively. The trachea was cannulated, and the rats were ventilated artificially with O2-enriched room air. Arterial blood gases were measured and maintained within the normal range by altering the ventilation rate and/or tidal volume. Rectal temperature was monitored and maintained within normal limits with a circulating-water heating blanket. Electrodes for recording lumbar sympathetic nerve activity (LSNA) were implanted by modification of a technique previously described (38). Through a midline abdominal incision, the lumbar sympathetic chain was identified and isolated immediately caudal to the left renal vein. Two Teflon-insulated silver wire electrodes (Medwire, 0.005 in. diameter, 36 gauge) threaded through silicone rubber tubing (0.025 in. ID) were placed around the isolated sympathetic chain. Nerves and electrodes were covered with polyvinylsiloxane gel (Cole-Parmer), which was allowed to harden before closure. A ground wire was attached to the exterior skin. Rats were placed in a Kopf stereotaxic frame, and the dorsal surface of the medulla was exposed through the atlanto-occipital membrane.

The arterial catheter was connected to a pressure transducer for recording of arterial pressure. Mean arterial pressure (MAP) was derived electronically using a low-pass filter. Heart rate (HR) was determined with a cardiotachometer triggered from the arterial pressure pulse. Sympathetic nerve activity was amplified 1,000 times using a preamplifier (model P511, Grass) and filtered using a band-pass frequency of 30 Hz–3 kHz. Action potentials were monitored with a Tektronix oscilloscope and an audio monitor (model M8, Grass). Nerve activity was rectified and integrated using a root-mean-square converter with a time constant of 28 ms. The rectified, integrated signal was averaged electronically. Background noise was defined as the residual signal from the nerve after the animal was euthanized. The amount of recorded nerve activity after subtraction of background noise was defined as LNSA.

Micromination. Multibarrel pipettes (5 or 7 barrels, outside tip diameter 40–80 µm) were placed unilaterally into the NTS under visual guidance using a Storz surgical microscope. Target stereotaxic coordinates for the NTS were 0.5 mm cranial and 0.5 mm lateral to calamus scriptorius and 0.5 mm ventral from the dorsal surface of the medulla. The initial criterion for accurate pipette placement within the NTS was a depressor and sympathoinhibitory response to pressure injection of 1 mM ACPD. If this criterion was not met, coordinates were adjusted, and the response to the agonist was retested. The average stereotaxic coordinates for pipette placement were 0.50 ± 0.02 mm cranial and 0.50 ± 0.01 mm lateral to calamus scriptorius and 0.50 ± 0.00 mm ventral from the dorsal surface of the medulla. Drugs were ejected from the pipette in volumes of 30 nl over a period of ~3 s by applying pulses of pressurized N2 to each barrel with use of a custom-constructed pressure ejection system. The volume of drug delivery was controlled by changing the injection pressure and/or duration of the pulse. The volume of the injection was determined by viewing the movement of the fluid meniscus in individual barrels of known internal diameter by using a microscope (×150) equipped with a calibrated eyepiece micrometer.

Experimental protocols. A range of concentrations (1 µM–10 mM) of DHPG was utilized to establish the dose-response relationship and dose of drug that produced 50% of the maximal response (ED50) of microinjections of DHPG in the NTS. All injections were made in equal volumes (30 nl), and the concentration of DHPG within the pipette was varied. Several concentrations of DHPG were injected into the same NTS location in each rat via a different barrel of the multibarrel pipette.

In some animals the effects of microinjection of DHPG and ACPD were compared. These two agonists have very similar potencies for group I mGluRs when tested using in vitro preparations (6, 7, 33). Unilateral NTS microinjection of 30 nl of equal concentrations (1 mM) of the agonists was performed, and changes in MAP, HR, and LSNA were recorded.

The effects of glutamate receptor antagonists on the response to DHPG and ACPD were evaluated. The general mGluR antagonist α-methyl-4-carboxyphenylglycine (MCPG, 10 mM) was administered for 1 min by repeated NTS microinjections of 30 nl every 10 s. Previously, MCPG given in this way was demonstrated to effectively block mGluRs, and this protocol does not produce nonspecific effects (12). Importantly, microinjection of saline with an identical procedure does not alter the response to NTS microinjection of glutamate (12). To eliminate differences in administration of antagonists, the ionotropic glutamate receptor antagonist kynurenic acid was administered in a similar manner (12). In addition, the ability of kynurenic acid to block the response to selective ionotropic glutamate receptors agonists kainic acid and N-methyl-D-aspartic acid (NMDA) was tested.

For experiments using glutamate receptor antagonists, the general protocol was as follows. Control responses to unilateral microinjection of 30 nl of an agonist were obtained. From a different pipette barrel, a glutamate receptor antagonist was then injected into the NTS, as described above. Immediately at the end of the 1-min period of antagonist injections, the agonist injection was repeated. In addition, agonist injections were repeated every 5 min after termination of antagonist administration to test for recovery from blockade. A minimum of 5 min between sequential injections of agonists was used to allow baseline parameters to return to preinjection levels. Preliminary studies demonstrated that a period of 5 min between agonist injections was adequate to prevent tachyphylaxis. Because of the difference in the time course of the effects of the antagonists (12, 29), a minimum of 45 and 15 min for recovery was allowed after injection of kynurenic acid and MCPG, respectively, before initiation of another experimental protocol. For the experiments using glutamate receptor antagonists, the concentrations of individual drugs were as follows: 1 mM DHPG, 1 mM ACPD, 500 µM NMDA, 100 µM kainic acid, 40 mM kynurenic acid, and 10 mM MCPG.

Histological analysis. In addition to functional identification by depressor and sympathoinhibitory responses to agonist injection, histological analysis was performed in some rats (n = 20) to confirm accurate pipette placement within the NTS. At the end of the experiment, a minimum of 30 nl (41 ± 4 nl) of 2% pontamine sky blue dye was ejected from a different pipette barrel to mark the injection site. After euthanasia the brains were removed and stored in 10% phosphate-buffered formalin that contained 3% sucrose. Frozen 40-µm coronal sections of the medulla were mounted on slides, and coverslips were attached.

Drugs. Kynurenic acid, urethan, kainic acid, and pontamine sky blue were obtained from Sigma Chemical (St. Louis, MO). ACPD and NMDA were obtained from Research Biochemicals International (Natick, MA). DHPG and MCPG were obtained from Tocris Cookson (St. Louis, MO). Drugs were dissolved in distilled water. Kynurenic acid and MCPG were first solubilized with NaOH (1 M) before dilution with...
vehicle. All drugs were adjusted to pH 7.2–7.6. Drug doses are expressed as the free base of each drug.

Data analysis. The LSNA responses to drug injections were analyzed as percentage of the baseline level of LSNA before control agonist or antagonist injections. The baseline level of LSNA was defined to be 100%.

A logistic curve was fit to the mean MAP, HR, and LSNA data from the dose-response experiments. A logarithmic scale was used for the doses of DHPG. The midpoint of the generated curve is the ED₅₀.

In some instances, the peak responses to agonists before and after kynurenic acid were calculated as a percent change to normalize the baseline change produced by blockade of ionotropic glutamate receptors. The percent change was computed by dividing the difference between the peak response to the agonist and the control value immediately before the agonist injection by the control value and then multiplying the total by 100.

Values are means ± SE. Data comparing levels of MAP, HR, or LSNA before and after agonist injections during control, antagonist administration, and recovery (5 min after antagonist administration) were analyzed by two-way ANOVA with repeated measures. Peak changes in MAP, HR, or LSNA in response to agonist injections during control, antagonist administration, and recovery (5 min after antagonist administration) were analyzed by one-way ANOVA with repeated measures. When ANOVA indicated a significant interaction, differences between individual means were assessed by a least significant difference test (35). The peak changes in MAP, HR, or LSNA produced by 1 mM ACPD and 1 mM DHPG were compared by Student's paired t-test. P < 0.05 was considered statistically significant. All statistical analyses were performed using Sigma Stat for Windows (Jandel Scientific, San Rafael, CA) software package.

RESULTS

Effect of activation of group I mGluRs. Unilateral NTS microinjection of DHPG to activate group I mGluRs produced decreases in MAP, HR, and LSNA. Representative examples of the response to 30 nl of 1 mM DHPG are presented in the control injections in Fig. 1. The cardiovascular effects of DHPG typically peaked within 30 s and required ~3 min to recover (Fig. 1, control injections). The MAP, HR, and LSNA responses to NTS microinjection of DHPG were dose dependent (Fig. 2). A concentration range of DHPG from 1 µM to 10 mM (0.03–300 pmol in 30 nl) was used. The ED₅₀ of DHPG for the decrease in MAP, HR, and LSNA was determined to be 57, 50, and 100 µM, respectively.

Comparison of DHPG and ACPD. Microinjection into the NTS of ACPD or DHPG produced qualitatively similar responses. In in vitro preparations, ACPD and DHPG have a nearly identical EC₅₀ for group I mGluRs (6, 7, 33). Therefore, responses to equal concentrations of DHPG and ACPD were compared in a subset of animals (n = 8). Injection of 30 nl of 1 mM DHPG into the NTS decreased MAP (Δ = −26 ± 2 mmHg), HR (Δ = −16 ± 4 beats/min), and LSNA (Δ = −21 ± 4% control). In the same eight animals, injection of 30 nl of 1 mM ACPD into the NTS decreased MAP (Δ = −24 ± 2 mmHg), HR (Δ = −31 ± 9 beats/min), and LSNA (Δ = −23 ± 6% control). The responses were not significantly different between the two mGluR agonists.

Effect of blockade of mGluRs. The purpose of this set of experiments was to determine whether the responses to DHPG and ACPD were mediated by activation of mGluRs. The effect of blockade of mGluRs on the response to 1 mM DHPG in the NTS in a single animal is illustrated in Fig. 1A. In this example, DHPG microinjection decreased MAP, HR, and LSNA. After MCPG the response to DHPG was abolished. Average (n = 6) cardiovascular parameters at baseline and after DHPG microinjection under control, MCPG, and recovery conditions are presented in Fig. 3. The peak changes in MAP, HR, and LSNA in response to microinjection of the mGluR group I agonist before and after MCPG are presented in Table 1. Control injections of DHPG...
decreased MAP, HR, and LSNA. Unilateral NTS blockade of mGluRs with MCPG did not alter baseline MAP, HR, or LSNA (Fig. 3, open bars). The MAP and LSNA response to DHPG was abolished after 1 min of MCPG administration. The inhibition of the peak change in HR due to injection of DHPG by MCPG failed to reach statistical significance (Table 1; \( P = 0.057 \)). All responses to DHPG had recovered by 5 min after termination of the MCPG administration.

In addition to DHPG, the ability of MCPG to block the response to the general mGluR agonist ACPD was assessed in nine animals. The average cardiovascular parameters before and after injection of ACPD during control, MCPG, and recovery are presented in Fig. 4. The peak changes produced by ACPD injection are presented in Table 1. Control injections of ACPD produced a decrease in MAP, HR, and LSNA. After application of MCPG for 1 min, the MAP, HR, and LSNA response to ACPD was abolished (n = 9). Five minutes after the end of the MCPG injections, the MAP, HR, and LSNA responses to ACPD were significant; however, the peak MAP response was still blunted compared with control (Table 1).

Effect of blockade of ionotropic glutamate receptors. In this set of experiments the purpose was to determine the potential involvement of ionotropic glutamate receptors in the response to DHPG or ACPD. Initially, the ability of kynurenic acid to block ionotropic glutamate receptors was verified. Unilateral blockade of ionotropic glutamate receptors in the NTS typically increased baseline MAP and LSNA by 5 min after the microinjection of 30 nl of various concentrations of DHPG. A range of concentrations (1 \( \mu \text{M} - 10 \text{ mM} \)) of DHPG was used. ● Measured responses (average of several experiments in increasing dose order: \( n = 3, 4, 5, 13, 9, 12, 17, \) and 5). Line through data points, derived dose-response curve. A logistic equation was utilized to fit data points and determine dose of drug that produced 50% of maximal response (ED\(_{50}\)). See Fig. 1 legend for definition of abbreviations.

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ANOVA and least significant difference (between baseline and peak response to ACPD determined by 2-way after end of MCPG injections (Rec, ylic acid (ACPD) during control (Con), immediately after NTS mGluRs in NTS abolished response to microinjection of ACPD. See Fig. 1 legend for definition of abbreviations.

Fig. 4. MAP, HR, and LSNA before (open bars) and after (filled bars) NTS microinjection of 1 mM 1-aminocyclopentane-1S,3R-dicarboxylic acid (ACP) during control (Con), immediately after NTS administration of 10 mM MCPG, and at recovery of responses 5 min after end of MCPG injections (Rec, n = 9). *Statistical difference between baseline and peak response to ACPD determined by 2-way ANOVA and least significant difference (P < 0.05). Blockade of mGluRs in NTS abolished response to microinjection of ACPD. See Fig. 1 legend for definition of abbreviations.

Table 1. Peak responses to microinjection of 1 mM DHPG or 1 mM ACPD into NTS before and after NTS administration of 10 mM MCPG or 40 mM KYN

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<th>MCPG (n = 6)</th>
<th>KYN (n = 9)</th>
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<tr>
<td></td>
<td>Control</td>
<td>0 min</td>
</tr>
<tr>
<td>Δ MAP, mmHg</td>
<td>-27 ± 6</td>
<td>-21 ± 6†</td>
</tr>
<tr>
<td>Δ HR, beats/min</td>
<td>-19 ± 7</td>
<td>-12 ± 2</td>
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<tr>
<td>Δ LSNA, %control</td>
<td>-16 ± 3</td>
<td>-5 ± 2‡</td>
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ACPD (n = 9)  

|                    | Control     | 0 min      | 5 min | Control     | 0 min | 5 min |
| Δ MAP, mmHg        | -29 ± 3     | -20 ± 3‡    | -21 ± 2 | -20 ± 3     | -29 ± 3‡    |
| Δ HR, beats/min    | -22 ± 6     | -16 ± 5‡    | -14 ± 3 | -23 ± 6     | -16 ± 4     |
| Δ LSNA, %control   | -20 ± 4     | -15 ± 3     | -12 ± 1 | -14 ± 2     | -19 ± 3‡    |

Values are means ± SE. DHPG, 3,5-dihydroxyphenylglycine; ACPD, 1-aminocyclopentane-1S,3R-dicarboxylic acid; MCPG, α-methyl-4-carboxyphenylglycine; KYN, kynurenic acid; MAP, mean arterial pressure; HR, heart rate; LSNA, lumbar sympathetic nerve activity. *P < 0.05 compared with control agonist responses produced before antagonist administration. †P < 0.05 compared with 0 min agonist response after antagonist.

Kynurenic acid injections, and these parameters returned to control levels by 10 min. Administration of kynurenic acid abolished the responses produced by microinjection of the selective ionotropic glutamate receptor agonists NMDA and kainic acid (Table 2). The responses to kainic acid and NMDA recovered by 21 and 38 min after kynurenic acid, respectively.

The effect of blockade of ionotropic glutamate receptors on the response to 1 mM DHPG in the NTS in a single animal is illustrated in Fig. 1B. In this representative animal, DHPG decreased MAP, HR, and LSNA. After kynurenic acid the response to DHPG was not attenuated. The average (n = 5) cardiovascular parameters before and after DHPG injection during control and immediately and 5 min after kynurenic acid administration are presented in Fig. 5. The peak changes in response to DHPG injection during control and after kynurenic acid are presented in Table 1. Under control conditions, injections of DHPG produced a decrease in MAP, HR, and LSNA. Administration of kynurenic acid did not alter the HR or LSNA response to DHPG (n = 5). The decrease in MAP in response to DHPG was potentiated at 5 min after the end of the kynurenic acid injections (Table 1). This potentiation of the depressor response to DHPG is most likely due to the baseline increase in MAP after kynurenic acid administration, because the depressor response was not significantly different after kynurenic acid when calculated as a percent change (−26 ± 2, −27 ± 4, and −31 ± 2% for control, 0 min, and 5 min, respectively).

In addition to DHPG, the effects of kynurenic acid on the response to the general mGluR agonist ACPD were assessed in nine animals. The peak cardiovascular changes produced by ACPD injection during control and immediately and 5 min after kynurenic acid administration are presented in Table 1. Control injections of ACPD produced a decrease in MAP, HR, and LSNA. Administration of kynurenic acid did not alter the HR response to ACPD (n = 9). The depressor and sympathoinhibitory responses to ACPD were potentiated at 5 min after the end of the kynurenic acid injections compared with control. These enhanced depressor and
sympathoinhibitory responses to ACPD may be due in part to the baseline increase in MAP and LSNA after kynurenic acid administration. When calculated as a percent change, the sympathoinhibitory (−12 ± 1, −14 ± 2, and −17 ± 3% for control, 0 min, and 5 min, respectively) and the depressor (−20 ± 2, −20 ± 3, and −27 ± 3% for control, 0 min, and 5 min, respectively) effects of ACPD at 5 min after kynurenic acid were reduced, but the responses were still significantly enhanced compared with control.

Histology. In all injection sites, ACPD or DHPG produced decreases in MAP, HR, and LSNA. Histological analysis of the injection sites marked with dye (n = 20) verified that all pipette tips were within the NTS. All the pipette locations were within the intermediate NTS, lateral to the area postrema and 500 µm rostral to calamus scriptorius (Fig. 6). The spread of 30 nl of dye was restricted to the intermediate NTS.

**DISCUSSION**

This study tested the hypothesis that activation of group I mGluRs produces cardiovascular effects consistent with excitation of NTS neurons involved in inhibition of HR and sympathetic outflow. The primary findings of the study were that microinjection of DHPG into the NTS produced dose-dependent decreases in MAP, HR, and LSNA. These effects of DHPG were similar to those of general mGluR activation with ACPD. The effects of DHPG or ACPD were abolished by administration of the mGluR antagonist MCPG. Furthermore, blockade of ionotropic glutamate receptors with kynurenic acid did not attenuate the effects of DHPG or ACPD. These data suggest that DHPG and ACPD activate mGluRs in the NTS to produce depressor, bradycardic, and sympathoinhibitory responses that do not require ionotropic glutamate receptors. Thus the cardiovascular response to activation of group I mGluRs in the NTS mimics stimulation of the arterial baroreflex and appears to produce this effect by exciting neurons within the NTS that lead to inhibition of sympathetic outflow and to decreases in HR.

The cardiovascular responses to DHPG or ACPD in the NTS likely are due to activation of mGluRs. Both agonists have been shown to be selective for mGluRs and devoid of actions at other, non-mGluR, receptors (7, 26). In the present study the cardiovascular effects of these agonists were abolished by administration of MCPG, which is an antagonist of group I and II mGluRs (6, 7, 25). Consistent with previous work utilizing ACPD (31), blockade of ionotropic glutamate receptors with kynurenic acid did not attenuate the

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**Table 2. Peak responses to microinjection of 500 µM NMDA and 100 µM kainic acid into NTS before and after NTS administration of 40 mM KYN**

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<th>Peak NMDA Response</th>
<th>Peak Kainic Acid Response</th>
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<tr>
<td></td>
<td>(n = 4)</td>
<td>(n = 4)</td>
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<tr>
<td>Δ MAP, mmHg</td>
<td>Control 0 min 5 min</td>
<td>Control 0 min 5 min</td>
</tr>
<tr>
<td>Δ HR, beats/min</td>
<td>Control 0 min 5 min</td>
<td>Control 0 min 5 min</td>
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<tr>
<td>Δ LSNA, % control</td>
<td>Control 0 min 5 min</td>
<td>Control 0 min 5 min</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ MAP, mmHg</td>
<td>−42±6 −3±2* −1±1*</td>
<td>−36±8 −5±2* −5±2*</td>
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<tr>
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<tr>
<td>Δ LSNA, % control</td>
<td>−38±9 −2±1* −1±0*</td>
<td>−40±10 −6±2* −7±5*</td>
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Values are means ± SE. NMDA, N-methyl-D-aspartic acid; see Table 1 footnote for definition of other abbreviations. *P < 0.05 compared with control agonist responses produced before antagonist administration.

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**Fig. 5.** MAP, HR, and LSNA before (open bars) and after (filled bars) NTS microinjection of 1 mM DHPG during control (Con) and immediately (0 min) and 5 min after NTS administration of 40 mM KYN (5 min, n = 5). *Statistical difference between baseline and peak response to DHPG determined by 2-way ANOVA and least significant difference differences between baseline values are indicated by horizontal bars (P < 0.05). †Significant main effect of DHPG determined by 2-way ANOVA (P < 0.05). Response to microinjection of DHPG was not attenuated by blockade of ionotropic glutamate receptors in NTS. See Fig. 1 legend for definition of abbreviations.
effects of either mGluR agonist. Furthermore, DHPG is selective for group I mGluRs (6, 15, 16, 26, 33) and devoid of actions at group II and group III mGluRs (16, 26, 33). Therefore, the effects of DHPG in the NTS appear to be mediated by group I mGluRs. The general mGluR agonist ACPD activates most identified mGluRs but with varying potency. Interestingly, ACPD and DHPG have nearly equivalent EC50 values for group I mGluRs when tested in similar in vitro experimental preparations (6, 7, 33). In the present study, ACPD produced decreases in MAP, HR, and LSNA that were abolished by administration of MCPG and not attenuated by kynurenic acid. Also, equal concentrations of DHPG and ACPD produced quantitatively similar responses. Because ACPD and DHPG are equipotent for group I mGluRs, it seems likely that the effects of ACPD in the NTS are mediated primarily by group I mGluRs.

MCPG has been described and utilized as an effective antagonist of group I/II mGluRs that is devoid of actions at other classes of receptors (6, 7, 15, 19, 25, 27, 32). Recently, MCPG has been suggested to be a noncompetitive antagonist of the NMDA subtype of ionotropic glutamate receptors under specific conditions in vitro (8). It is unknown whether these circumstances occur in vivo. Importantly, the results from the present study do not support the concept that the effects of MCPG on the responses to DHPG and ACPD are due to antagonism of NMDA receptors in the NTS. Administration of kynurenic acid at a dose that totally eliminated the response to injection of NMDA into the NTS did not attenuate the effects of DHPG or ACPD. Thus antagonism of NMDA receptors cannot account for the effect of MCPG to abolish the response to DHPG or ACPD. In addition, the effects of MCPG on the responses to DHPG and ACPD cannot be accounted for by the method of administration. In the present study, kynurenic acid, which was microinjected by an identical protocol, did not attenuate the response to DHPG or ACPD. In a previous study, saline injected in a manner identical to that used for MCPG injection did not alter the response to microinjection of glutamate (12). Therefore, the ability of MCPG to abolish the responses to DHPG and ACPD likely is due to blockade of mGluRs within the NTS and not to a nonspecific action.

Administration of kynurenic acid did not attenuate the responses to DHPG or ACPD in the present study, suggesting that ionotropic glutamate receptors are not required for the responses to these mGluR agonists. Kynurenic acid has been utilized as a broad-spectrum antagonist specific for the ionotropic glutamate receptors (10, 14, 29, 31, 37). In the present study, kynurenic acid abolished the response to two different ionotropic agonists consistent with it being an effective ionotropic receptor antagonist. In other studies, microinjection of kynurenic acid into the NTS has been shown to block the function of the arterial baroreflex (29, 31, 37). Also, microinjection of kynurenic acid into the NTS has blocked the effects of ionotropic agonists and did not attenuate the response to ACh (29, 37). Interestingly, some of the cardiovascular responses to DHPG and ACPD were enhanced by blockade of ionotropic glutamate receptors. Although this potentiation of mGluR effects is due at least in part to the baseline increase in MAP and LSNA produced by kynurenic acid administration, it is possible that there is a direct or indirect tonic inhibition of mGluR effects by ionotropic glutamate receptors.

Consistent with the results from the present study, excitatory effects of mGluRs on NTS neurons have been reported in in vitro and in vivo studies (17, 18, 40). In the NTS brain slice preparation, ACPD produces several postsynaptic excitatory responses, including direct activation of NTS neurons, facilitation of the response to an ionotropic glutamate receptor agonist, and inhibition of the outward current produced by a GABA\textsubscript{A} agonist (17, 18). The direct depolarization and the attenuation of GABA\textsubscript{A} currents produced by ACPD are mimicked by tetanic stimulation of the tractus solitarius, suggesting that these effects can be produced by release of endogenous transmitter (17, 18). Blockade of mGluRs with MCPG inhibits the ability of ACPD to facilitate responses to ionotropic glutamate receptors and to attenuate GABA\textsubscript{A} effects (20). In vivo studies using anesthetized rats, iontophoresis of ACPD increases the firing rate of NTS neurons that receive baroreceptor input (40). The excitatory effect of ACPD on NTS neurons tends to be greater at neurons receiving monosynaptic input than at neurons receiving polysynaptic input. Taken together, these data support
the idea that mGluRs within the NTS produce postsynaptic excitatory responses on neurons that regulate MAP, HR, and LSNA. Data from the present study suggest that these excitatory effects may be mediated, at least in part, by group I mGluRs.

In addition to eliciting excitatory, postsynaptic effects, ACPD decreases evoked excitatory postsynaptic potentials via a presynaptic mechanism in the NTS brain slice preparation (17). Other studies have investigated the modulation of neurotransmitter release from baroreceptor neurons isolated from the nodose ganglion (22, 23). Activation of mGluRs in these cells decreases the activity of voltage-gated Ca\(^{2+}\) channels, which is consistent with an action of mGluRs to inhibit transmitter release (23). Also, activation of mGluRs attenuates vesicle exocytosis from baroreceptor afferents (22). Taken together, these studies support the concept that mGluRs presynaptically inhibit neurotransmitter release from baroreceptor afferents. Although the data from the present study do not support the idea that mGluRs inhibit transmitter release, they are not inconsistent with this concept. First, a postsynaptic excitatory effect most likely would mask any presynaptic reduction in glutamate release from baroreceptor afferents. In addition, it is probable that the population of presynaptic inhibitory mGluRs does not represent group I mGluRs. In other systems, group II and III mGluRs typically are presynaptic and inhibit neurotransmitter release (13). This idea is supported by an anatomic study that utilized immunohistochemical techniques to localize mGluRs within the NTS (24). A group I (mGluR1a) mGluR was identified on cell bodies within the NTS, whereas group II (mGluR2/3) and III (mGluR7) mGluRs were identified primarily on fibers in this region. These data are consistent with studies in other regions of the brain that have identified postsynaptic group I mGluRs (3, 34). Therefore, we speculate that mGluRs other than mGluR1a are involved in the presynaptic inhibition of neurotransmitter release from baroreceptor afferents in the NTS. Further studies with group II- or III-selective mGluR agonists and antagonists are needed to address this point.

The data from the present study suggest that group I mGluRs in the NTS produce cardiovascular responses that are consistent with activation of NTS neurons that inhibit MAP, HR, and LSNA. In addition, these effects of group I mGluRs do not require ionotropic glutamate receptors. On the basis of previous studies, several mechanisms could explain the effects of the group I mGluRs in the NTS. A model depicting these possibilities is presented in Fig. 7. This model includes a neuron in the NTS that is involved in regulation of sympathetic nerve activity, HR, and arterial pressure. The represented NTS neuron may receive primary afferent input, or it could be farther downstream within the NTS. The present data do not allow differentiation among specific neurons in the NTS. This NTS neuron is the target of several different projections, including a glutamatergic (e.g., baroreceptor afferent), an inhibitory (e.g., GABA interneuron), and an excitatory, nonglutamatergic (e.g., adrenergic, substance P, neuropeptide Y) input. The nonglutamatergic input may be an independent pathway, or it could be a coreleased transmitter from a glutamatergic projection. The inputs have been presented as separate pathways for clarity. One possible mechanism that could account for the results from this study is that the response to activation of group I mGluRs in the NTS could be due to direct activation of NTS neurons. It has been demonstrated that ACPD depolarizes NTS neurons via closure of K\(^+\) channels.
activity on NTS neurons. This effect of group I mGluRs during periods of elevated or prolonged afferent activity could be due to several possible mechanisms, including direct neuronal activation, potentiation of an excitatory synapse, HR, and arterial pressure. In summary, activation of group I mGluRs in the NTS by microinjection of DHPG produces depressor, bradycardic, and sympathoinhibitory responses. These responses are abolished by blockade of mGluRs with MCPG and are not attenuated by antagonism of ionotropic glutamate receptors with kynurenic acid. These data suggest that activation of group I mGluRs in the NTS produces cardiovascular responses consistent with activation of neurons involved in inhibition of sympathetic nerve activity, HR, and arterial pressure. These effects could be due to several possible mechanisms, including direct neuronal activation, potentiation of an excitatory input, or inhibition of GABA effects. Also in this study, equal concentrations of DHPG and the general mGluR agonist ACPD produced similar responses. These results in conjunction with their described pharmacology suggest that the effects of ACPD microinjected in the NTS are mediated primarily by group I mGluRs.

Perspectives

An important question is whether the group I mGluRs influence cardiovascular reflex function. Baroreceptor afferents are believed to release glutamate from their terminations in the NTS (29, 31, 37). Arterial baroreflex function is abolished by blockade of ionotropic glutamate receptors (29, 37). Thus group I mGluRs are not capable of sustaining baroreflex function in the absence of ionotropic glutamate receptors. In the hippocampus and cerebellum, mGluR1a is located on postsynaptic membranes and concentrated perisynaptic to excitatory synapses (3). If the group I mGluRs are located at extrasynaptic locations and activated by endogenous glutamate, then they may be involved during periods of elevated or prolonged afferent activity, and their action could enhance the effects of afferent activity on NTS neurons. This effect of group I mGluRs may contribute to the normal gain of the baroreflex to physiological increases in arterial pressure, or they may contribute to pathophysiological shifts in baroreflex function. In addition, the group I mGluRs may be involved in projections to the NTS that modulate baroreflex function. If the group I mGluRs enhance NTS neuronal excitability, then they may be involved in the regulation of sympathetic neurotransmission. Although enhancement of a nonglutamatergic excitatory pathway could explain the response to activation of group I mGluRs in the NTS, to our knowledge, there are no data that directly support such a concept. Taken together, it seems likely that group I mGluRs produce their responses by direct activation or by increasing the excitability of NTS neurons that are involved in regulation of sympathetic nerve activity, HR, and arterial pressure.

REFERENCES

12. Foley, C. M., J. A. Moffitt, M. Hay, and E. M. Hasser. Glutamate in the nucleus tractus solitarius activates both iono-